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ACTIVATION MECHANISM OF TRIS(2,3-DIBROMOPROPYL)PHOSPHATE TO THE POTENT MUTAGEN, 2-BROMOACROLEIN

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The potent mutagen 2-bromoacrolein is formed from the carcinogenic flame retardant tris(2,3-dibromopropyl)phosphate (Tris-BP) on incubation with hepatic microsomes. Substitution of deuterium for hydrogen at the terminal carbon atoms (C-3) of Tris-BP significantly decreased both the mutagenic response and the formation rate of 2-bromoacrolein. Mass spectral analysis of the 2-bromoacrolein that was formed from the selectively deuterated analogs of Tris-BP revealed that the primary mechanism for the formation of 2-bromoacrolein involves an initial oxidative dehalogenation at C-3 followed by a β-elimination reaction.

Tris(2,3-dibromopropyl)phosphate (Tris-BP), a halogenated alkyl phosphate, was used extensively as a flame retardant in synthetic fibers, most notably in children's sleepwear, until it was shown to be highly mutagenic to Salmonella typhimurium (1,2). Since that discovery, Tris-BP has been shown to be carcinogenic (3,4) and to cause acute renal tubular necrosis (5) in rats. Microsomal, NADPH-dependent oxidative metabolism is necessary for converting Tris-BP to mutagenic products (6). Although a recent report has implicated 2-bromoacrolein as a metabolite of Tris-BP (7), this compound has neither been detected as a metabolite nor has its mechanism of formation been established. We now report direct detection of 2-bromoacrolein as an oxidative, microsomal metabolite of Tris-BP. We also describe mechanisms for its formation based on results of comparative mutagenicities of selectively deuterated analogs of Tris-BP and an analysis of the deuterium content in the 2-bromoacrolein that is formed from these analogs.

MATERIALS AND METHODS

Propargyl alcohol, lithium aluminum hydride (LAH), phosphorus oxychloride, and pyridine were purchased from Aldrich Chemical Co. Lithium aluminum

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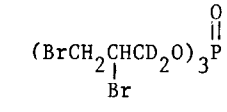
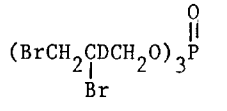
deuteride (LAD) and D_2O (>99.5% purity) were obtained form Stohler Isotope Chemicals. NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. Silica gel was obtained from MC/B subsidiary of E. Merck and Co. Salmonella typhimurium strain TA 100 was a gift from Dr. Bruce Ames.

Synthesis of Deuterium-Labeled Substrates. The specifically deuterated Tris-BP analogs (A,B,C) were prepared by acylation of the respectively deuterated 2,3-dibromopropanols with phosphorus oxychloride. The labeled 2,3-dibromopropanols were prepared from specifically deuterated allyl alcohols that were synthesized by published methods (8,9). To ice-cooled solutions of the appropriately deuterated allyl alcohol (3.05 g, 0.06 mol) in 10 ml CCl_4 was added dropwise liquid bromine (9.25 g, 0.06 mol) in 10 ml CCl_4. After complete addition at 0°-5°C the reaction was stirred at room temp. for 15 minutes, and then evaporated under reduced pressure to yield the desired brominated propanols. Purification was achieved by vacuum distillation of the crude products (b.p. 38-39°C at 0.3 mm Hg) to give approximately 9.3 g of product (72% yield). Deuterium incorporation was greater than 98% for each compound as determined by mass spectrometric analysis of the protonated molecular ion species generated by chemical ionization with methane as the reagent gas.



(A)



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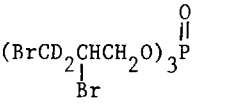

(C)

Mutagenesis Assays. Conditions for these assays have been described previously (6).

Metabolite Assays. Microsomes were prepared from the livers of male Sprague-Dawley rats, 180-200 g (Tyler Laboratories, Redmond, WA) and Tris-BP and its deuterated analogs were incubated as previously described (5). Aliquots (3 µl) of ethyl acetate extracts of the incubation mixtures were analyzed by gas chromatography-mass spectrometry (GC-MS) on a VG 70-70H mass spectrometer, equipped with a Hewlett-Packard 5710A gas chromatograph, and on-line to a VG Model 2035 data system. Analyses were performed in the EI mode using an accelerating voltage of 4 KV, an electron energy of 70 eV, and a trap current of 100 µA. Scans over the mass range 150-100 Dalton were taken repetitively at a scan rate of 1 sec/decade. The GC was equipped with a 30 m X 0.32 mm i.d. fused silica column coated with DB-5 as a stationary phase (J and W Scientific). Analysis of 2-bromoacrolein was performed using the following conditions: carrier gas, He (head pressure, 15 psi); injector temperature, 250°C; temperature program, splitless injection at oven temperature of 50°C, then temperature raised after 5 min by 5°/min to 150°C; GC interface temperature, 200°C; ion source temperature 200°C. Retention time of 2-bromoacrolein was approximately 7.5 min or 10.5 methylene units (MU) when measured relative to a homologous series of n-alkanes.

RESULTS AND DISCUSSION

Mutagenesis studies (Fig. 1) showed that the deuterated analogs A and B possessed mutagenic activity essentially equivalent to unlabeled Tris-BP. In marked contrast, analog C was much less active as a mutagen. In all cases, mutagenesis required incubation with a microsomal activation system. Thus,

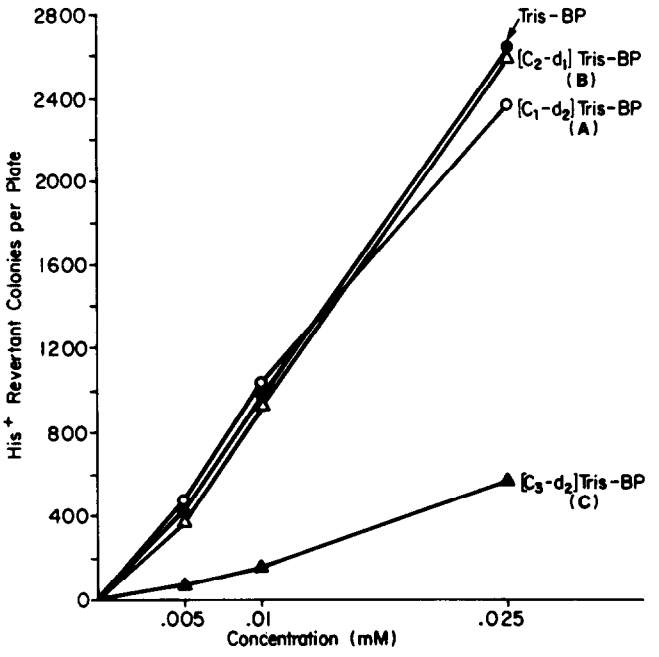

Fig. 1. Mutagenic activity of Tris-BP and its specifically deuterated analogs (A,B,C), assayed with Salmonella typhimurium strain TA 100 sensitive to base-pair substitution mutagens (16). Assays were performed as described in MATERIALS AND METHODS. Values are averages of duplicate determinations from 2 plates (2 incubations) after subtraction of the number of spontaneous reversion colonies (61-66 His^+ revertants/plate). The highest concentrations of substrates that were tested produced less than 25% bacterial cytotoxicity. Values were not significantly above background either in the absence of microsomes or NADPH.

substitution of deuterium for hydrogen at the terminal carbon atom (C-3) of Tris-BP apparently slows the rate of activation of the promutagen to the reactive mutagenic species.

Inasmuch as significant deuterium isotope effects have been observed on oxidative dehalogenation by cytochrome P-450 (10), we speculated that such a mechanism was involved in the oxidative activation of Tris-BP (Fig. 2). Because a significant isotope effect on mutagenicity was only observed with deuterium substitution at C-3, we propose that this is the major site of oxidation that leads to formation of the mutagen (Pathway I). Dehydrobromination of an initially formed gem-bromohydrin would produce an α-bromoaldehyde which would be expected to dehydrophosphorylate by a β-elimination reaction to produce 2-bromoacrolein and bis(2,3-dibromopropyl)phosphate (Bis-BP). 2-Bromoacrolein is a potent direct-acting mutagen (11) which in

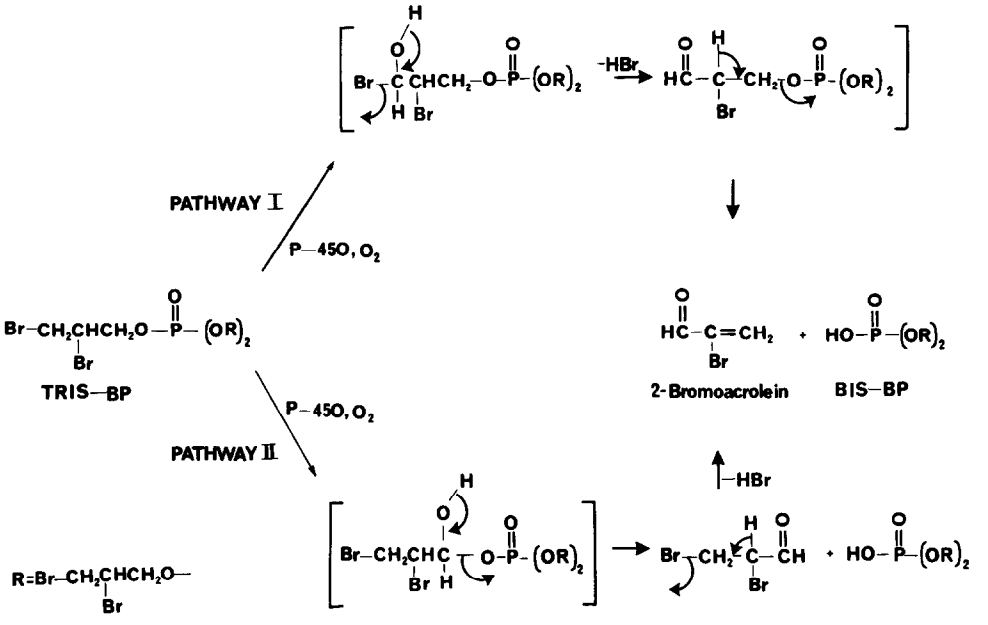

Fig. 2. Proposed pathways for the metabolic activation of Tris-BP to the mutagen 2-bromoacrolein. Both bromide and Bis-BP have been identified as microsomal metabolites of Tris-BP (17-19).

our assays produced over 4000 revertant colonies at 10 μM concentrations. Alternatively (Pathway II), 2-bromoacrolein could be formed by initial oxidation at C-1 followed by dehydrophosphorylation to 2,3-dibromopropanal and Bis-BP. This reactive aldehyde is known to dehydrobrominate to form 2-bromoacrolein (7).

In order to substantiate some of the features of the proposed pathway, incubation mixtures of Tris-BP and its deuterated analogs (A,B,C) with rat liver microsomes were analyzed for the presence of 2-bromoacrolein by gas chromatography/mass spectrometry. Results of these studies (Fig. 3) clearly demonstrated that 2-bromoacrolein was formed in an NADPH-dependent reaction by rat liver microsomes. Consistent with the significantly lower mutagenic activity displayed by analog C, its rate of metabolism to 2-bromoacrolein was approximately one-third the rate of metabolism of Tris-BP or analogs A and B to 2-bromoacrolein.

Furthermore, analysis of deuterium content in 2-bromoacrolein formed from analog A showed that approximately 80% of the product contained two deu-

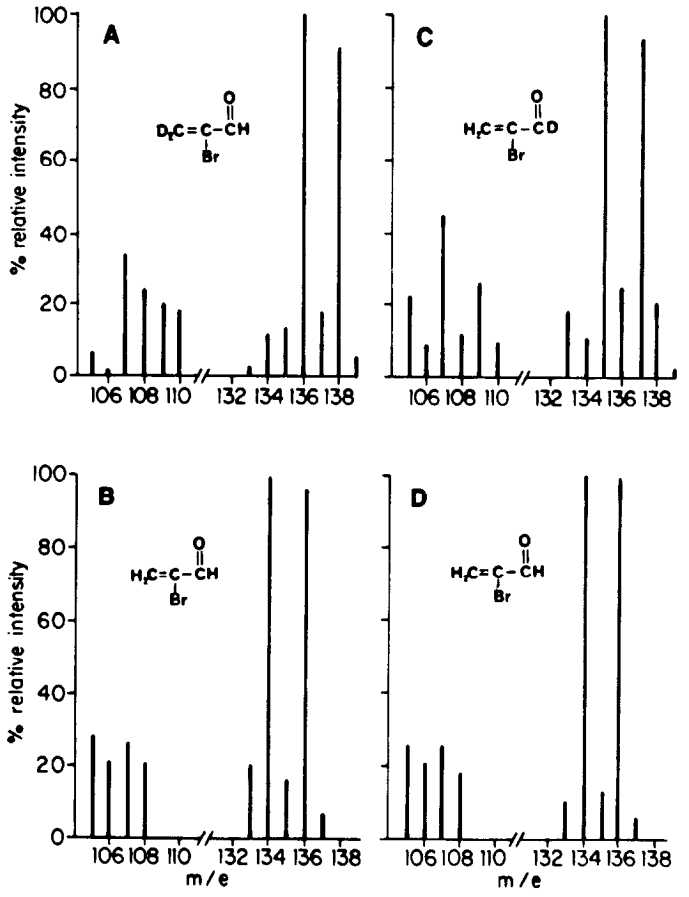

Fig. 3. Mass spectra of the parent ions and ions resulting from the loss of the formyl radical and carbon monoxide from 2-bromoacrolein that was formed as a metabolite of (A) [C_1-d_2] Tris-BP, (B) [C_2-d_1] Tris-BP, (C) [C_3-d_2] Tris-BP, and (D) Tris-BP. Gas chromatography-mass spectrometry (GC-MS) was carried out as described in MATERIALS AND METHODS.

terium atoms.[1] This rules out oxidation at C-1 as the major reaction involved in the formation of 2-bromoacrolein from Tris-BP. Analysis of the deuterium content in 2-bromoacrolein produced from analog B showed complete loss of the C-2 deuterium, as would be expected. Finally, analysis of the deuterium content from analog C showed 2-bromoacrolein as a metabolite with the retention of one deuterium atom. The deuterium atom was located on the aldehyde carbon atom as evidenced by the M-2 ion at m/z 133, nominally

[1] These values must be considered as only approximate in that we do not have synthetic standards of the deuterated bromoacroleins which would be necessary for accurate analyses of deuterium content. However, this does not alter our conclusion about the major pathway involved in 2-bromoacrolein formation from Tris-BP.

visualized as the acylonium ion E, and by the M-30 ions at m/z 105 and 107 indicating loss of the CDO radical with resulting formation of ion F. Loss of CO produces ions at m/z 107 and 109.

(E) (F)

Approximately 28% of 2-bromoacrolein appears to be formed from analog C with retention of two deuterium atoms based on the ion intensities at m/z 136 and 138.[1] This could possibly result from switching of metabolic pathways so that more oxidation occurs at C-1. Other oxidations that are catalyzed by cytochrome P-450 have been found to be subject to switching of oxidation away from sites of deuterium incorporation (12,13).

Thus, under the conditions of our assays, mutagenicity of the flame retardant Tris-BP primarily results from oxidation at C-3 (Pathway I, Figure 2) which results in the formation of the potent direct acting mutagen, 2-bromoacrolein, although details of the reaction mechanism remain to be investigated. The significance of this reaction in the carcinogenic and nephrotoxic actions of Tris-BP *in vivo* requires further study inasmuch as other toxic metabolites of Tris-BP have been detected *in vivo* (14,15).

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REFERENCES

1. Blum, A. and Ames, B. N. (1977) Science 195, 17-23.

2. Prival, M. J., McCoy, E. C., Gutter, B. and Rosenkranz, H. S. (1977) Science 195, 76-78.

3. National Cancer Institute Carcinogenesis Testing Program (1978) National Cancer Institute Carcinogenesis Technical Report, Series No. 76, Washington, D.C.

4. Reznik, G., Ward, J. M., Hardisty, J. F.and Russfield, A. (1979) J. Natl. Cancer Inst. 63, 205-212.

5. Soderlund, E., Dybing, E. and Nelson, S. D. (1980) Toxicol. Appl. Pharmacol. 56, 171-181.

6. Soderlund, E., Nelson, S. D. and Dybing, E. (1979) Acta Pharmacol. Toxicol. 45, 112-121.

7. Marsden, P. J. and Casida, J. E. (1982) J. Agric. Food Chem. 30, 627-631.

8. Schuetz, R. D. and Millard, F. W. (1959) J. Org. Chem. 24, 297-300.

9. McMichael, K. D. (1967) J. Amer. Chem. Soc. 89, 2943-2947.

10. Pohl, L. R. and Krishna, G. (1978) Life Sci. 23, 1067-1072.

11. Rosen, J. D., Segall, Y. and Casida, J. E. (1980) Mutat. Res. 78, 113-119.

12. Horning, M. G., Haegele, K. D., Sommer, K. R., Nowlin, J., Stafford, M. and Thenot, J-P. (1976) Proceedings of the Second International Conference on Stable Isotopes, pp. 41-54, NTIS, Springfield.

13. Farmer, P. B., Foster, A. B., Jarman, M., Kiburis, J. H., Newell, D. R. and Reed. D. J. (1979) Stable Isotopes: Proceedings of the Third International Symposium, pp. 371-378, Academic Press, New York.

14. Blum A., Gold, M. D., Ames, B. N., Kenyon, C., Jones, F. R., Hett, E. A., Dougherty, R. C., Horning, E. C., Dzidic, I., Carrol, D. I., Stillwell, R. N. and Thenot, J-P. (1979) Science 201, 1020-1023.

15. Lynn, R. K., Garvie-Gould, C., Wong, K. and Kennish, J. M. (1982) Toxicol. Appl. Pharmacol. 63, 105-119.

16. Ames, B. N., McCann, J. and Yamasaki, E. (1975) Mutat. Res. 31, 347-364.

17. Lynn, R. K., Wong, K., Dickinson, R. G., Gerber, N. and Kennish, J. M. (1980) Res. Commun. Chem. Pathol. Pharmacol. 28, 351-360.

18. Nomeir, A. and Matthews. H. B. (1983) Toxicol. Appl. Pharmacol. 67, 357-369.

19. Soderlund, E. J., Nelson, S. D., Omichinski, J. G., Gordon, W. P. and Dybing, E. (1984) Biochem. Pharmacol., in press.